

Viscous Fingering-like Instability of Cell Fragments

A. C. Callan-Jones,¹ J.-F. Joanny,¹ and J. Prost^{1,2}

¹*Physicochimie Curie (CNRS-UMR168),*

Institut Curie, Section de Recherche,

26 rue d'Ulm 75248 Paris Cedex 05 France

²*E.S.P.C.I., 10 rue Vauquelin, 75231 Paris Cedex 05, France*

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Abstract

We present a novel flow instability that can arise in thin films of cytoskeletal fluids if the friction with the substrate on which the film lies is sufficiently strong. We consider a two dimensional, membrane-bound fragment containing actin filaments that is perturbed from its initially circular state, where actin polymerizes at the edge and flows radially inward while depolymerizing in the fragment. Performing a linear stability analysis of the initial state due to perturbations of the fragment boundary, we find, in the limit of very large friction, that the perturbed actin velocity and pressure fields obey the very same laws governing the viscous fingering instability of an interface between immiscible fluids in a Hele-Shaw cell. A feature of this instability that is remarkable in the context of cell motility, is that its existence is independent of the strength of the interaction between cytoskeletal filaments and myosin motors, and moreover that it is completely driven by the free energy of actin polymerization at the fragment edge.

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Directed motion and shape change allow cells to respond to their environment and play central roles in many biological processes such as embryonic development, wound healing, and formation of cancer metastases. Almost universally, crawling of cells on a surface or extracellular matrix involves the protrusion of a thin leading edge, the lamellipodium, driven by the polymerization of the actin cytoskeleton, the adhesion to the substrate via specific proteins and molecular motor-enabled contraction of the cytoskeleton to translocate the trailing cell body [1]. Remarkably, physical units far simpler than eukaryotic cells can display self-sustained motion: the work of Ref. [2] shows that nearly flat cell fragments, containing only actin cytoskeleton and myosin II motors enclosed by a plasma membrane, are able to perform polymerization/contraction-driven motion. Furthermore the fragments can spontaneously switch between motile and non-motile states. The observations of Ref. [2] have led us to study theoretically actin-driven motility and shape dynamics in these simpler systems with few structural elements and few measurable parameters.

The actin cytoskeleton is a highly complex medium: it is polar as actin polymerizes at its “plus” end, facing the membrane abutting the lamellipodium; it is viscoelastic; and it is active and driven out of equilibrium by ATP hydrolysis, needed for continuous polymerization (treadmilling) and to generate myosin motor-induced stresses. Recently, a generic hydrodynamic theory has been developed to describe active, polar media [3]. Using a small number of phenomenological parameters, it can account for a number of motility phenomena due to the coupling of actin filaments to myosin activity [4, 5, 6]. The interaction of the cell with its environment is also very important in describing motility; for example, it has been demonstrated that cells crawling on a heterogeneous substrate tend to migrate to regions of greater substrate adhesion [7] and greater substrate rigidity [8]. Cytoskeletal actin in a cell or cell fragment is able to transmit forces to its substrate through transmembrane proteins [8, 9, 10], namely integrins, which bind reversibly to the substrate. In general, integrins cluster to form focal adhesions whose size and mechanical properties are determined by chemical and mechanical cues and can regulate the force that they exert. If, however, the actin velocity relative to the substrate is small compared to a/τ , where a is a molecular size and τ is the average time during which an integrin remains bound, then the force exerted by the moving filaments on the substrate can be expressed as a friction force, proportional to the actin velocity [5, 11, 12, 13].

In this Letter, we demonstrate that polymerization and large friction forces are sufficient

to destabilize an initially stationary, circular cell fragment. We start by considering a very simplified model of actin cytoskeletal flow in a cell fragment, as shown schematically in Fig. 1. The fragment is very thin and there is no flow or any spatial dependence in the z -direction; also we consider that the thickness of the fragment is constant. We further assume that the material in the fragment can be modeled as a single fluid: this implies that we treat only the flow of actin, and assume that the cytosol (including free actin monomers) is stationary relative to the substrate. Furthermore, we treat the cytoskeleton as an incompressible liquid, ignoring the elastic response that occurs on times shorter than the viscoelastic relaxation time.

Actin polymerization is regulated by proteins such as those of the Wiskott-Aldrich syndrome family (WASP), which localize in the cell membrane at the fragment edge [14]. For the purposes of this work, it is sufficient to assume that actin polymerizes only at the fragment edge and in the direction normal to the boundary. Newly polymerized actin flows away from the fragment edge by treadmilling, due the turnover of free actin monomers back to the edge for further polymerization that is enabled by actin depolymerization in the bulk. For simplicity, we assume that the filament depolymerization is spatially uniform and occurs at a rate proportional to the filament density.

These simplifications imply that the actin flow in the unperturbed, circular state induced by localized polymerization and uniform depolymerization is imposed by the continuity equation $\nabla \cdot (\rho \mathbf{v}_0) = -k_d \rho$, where ρ is the actin filament density and k_d is the depolymerization rate. The assumption of incompressibility directly leads to the radially-directed treadmilling speed

$$v_0 = -\frac{k_d}{2}r. \quad (1)$$

Note that in the stationary state continuity requires that $\frac{k_d}{2}R_0 = v_p$, where R_0 is the unperturbed fragment radius and v_p is the polymerization velocity.

The cytoskeleton dynamics is described by the hydrodynamic equations for active polar gels of Ref. [3], which themselves are a generalization of the hydrodynamics of liquid crystals [15, 16] modified to account for the coupling between stresses and active motors as well as actin polarization and motors. We can, however, proceed by considering that the friction with the substrate only couples directly to the actin flow and not to the polarization. The viscous fingering instability that will be seen shortly to be driven by edge polymerization

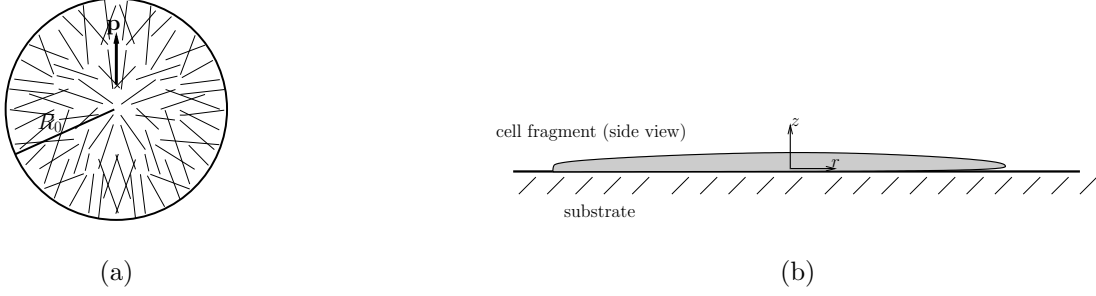


FIG. 1: (a) Schematic drawing of actin cytoskeleton in the unperturbed, radial state. The direction of average actin polarization is radial, and is indicated by $\mathbf{p} = \hat{\mathbf{r}}$. (b) A side view of the fragment.

and bulk depolymerization in the large friction limit can then be most easily illustrated by neglecting the dynamics of the polarization field, \mathbf{p} , and assuming this quantity is fixed along the radial direction so that $\mathbf{p} = \hat{\mathbf{r}}$ throughout.

Ignoring the dynamics of the polarization field, the constitutive laws of Ref. [3] reduce to those for an isotropic, viscous fluid of viscosity η , augmented by an active term in the deviatoric stress component σ_{rr} that reflects the myosin-mediated interaction between actin filaments that are nearly aligned; that is, $\underline{\underline{\sigma}} = 2\eta\underline{\underline{u}} - \zeta\Delta\mu\hat{\mathbf{r}}\hat{\mathbf{r}}$, where η is the actin viscosity; $\underline{\underline{u}}$ is the velocity gradient tensor; $\Delta\mu$ is the chemical potential difference between ATP and its hydrolysis products; and where for contractile motors the activity coefficient ζ is negative [17]. The constitutive laws are completed at low Reynolds numbers by the force balance $\nabla \cdot \underline{\underline{\sigma}} = \nabla P + \xi \mathbf{v}$, where ξ is the friction coefficient between the cytoskeletal filaments and the substrate. Scaling lengths by R_0 , times by $1/k_d$, and stresses and pressures by ηk_d (keeping the same variable names for the new, dimensionless quantities), it follows that viscosity and friction affect the cytoskeletal dynamics through the dimensionless parameter $\lambda^2 = \frac{\eta}{\xi R_0^2}$. In the limit of very large friction, that is, $\lambda \rightarrow 0$, the leading term in the force balance is simply

$$\mathbf{v} \sim -\lambda^2 \nabla P, \quad (2)$$

The velocity satisfies a two dimensional Darcy's law, as it would for the flow in a Hele-Shaw cell [18]. Based on the available experimental results we find that the quantity λ^2 is in fact quite small. Taking a value of $\xi \simeq 10^5 \text{ Pa}\cdot\text{s}/\mu^2$ [19], $\eta \simeq 10^4 \text{ Pa}\cdot\text{s}$ [12, 20], and $R_0 \simeq 10 \mu\text{m}$ we find $\lambda^2 \simeq 10^{-3}$.

We now perturb the edge of the fragment, so that in terms of the polar angle θ the

fragment edge is now at a position $R(\theta, t) = 1 + \delta R(\theta, t)$. For $\delta R(\theta, t) \ll 1$, we perform a linear stability analysis by writing $\delta R(\theta, t) = \sum_{m=1}^{\infty} \delta R_m(t) \cos(m\theta)$, and similarly for the two components of the perturbed velocity field, $\delta v_r = \sum_{m=1}^{\infty} \delta v_{r,m}(r, t) \cos(m\theta)$ and $\delta v_\theta = \sum_{m=1}^{\infty} \delta v_{\theta,m}(r, t) \sin(m\theta)$, and the pressure field $\delta P = \sum_{m=1}^{\infty} \delta P_m(r, t) \cos(m\theta)$. The $m = 0$ mode is excluded from this discussion since it is trivially stable because the quantity of actin in the fragment is fixed. In assuming that the depolymerization rate does not change as a result of the perturbation and that the filament density remains unchanged, it follows that $\nabla \cdot \delta \mathbf{v} = 0$ and therefore $\delta v_{\theta,m} = -\frac{1}{m} \frac{d(r\delta v_{r,m})}{dr}$. Applying Eq. (2) to the perturbed quantities $\delta \mathbf{v}$ and δP , we find that $\nabla^2 \delta P = 0$ and therefore $\delta P_m(r) \sim r^m$ and $\delta v_{r,m}(r, t) = -\delta v_{\theta,m}(r, t) = A_m(t)r^{m-1}$. The coefficient $A_m(t)$ can be found by imposing the force free condition at the boundary, namely

$$\delta P_m|_{r=1} + \delta R_m \frac{dP_0}{dr} \Big|_{r=1} = 0, \quad (3)$$

leading to $A_m(t) = \frac{m}{2} \delta R_m(t)$. The growth rate of the perturbation modes $\delta R_m(t)$ is obtained by noting that, to linear order in δR ,

$$\frac{d\delta R_m}{dt} \approx \delta v_{r,m}(R_0) + \delta R_m \frac{dv_0}{dr}, \quad (4)$$

which, using the expression for $A_m(t)$, gives $d\delta R_m/dt = \omega_m \delta R_m$, where the leading order growth rate, in units of k_d , is

$$\omega_m \sim \frac{m-1}{2} + O(\lambda^2). \quad (5)$$

Note that the mode $m = 1$, corresponding to an infinitesimal translation of the circular fragment, is marginally stable, as required by translational symmetry.

The linear dispersion relation, ω_m , is a common feature to a number of Laplacian growth problems, for example the viscous fingering instability that occurs at an interface between two immiscible liquids in a Hele–Shaw cell [21]. The physics of the instability is understood as follows. The pressure gradient at the edge is $dP_0(r=1)/dr > 0$, and a perturbation with $\delta R < 0$, for example, requires a perturbed pressure $\delta P(r=1) > 0$ to keep the boundary force-free, to leading order in λ^2 . An excess pressure at the edge relative to the fragment center ($\delta P(r=0) = 0$ for $m > 0$) drives an inward-directed flow, thus amplifying the initial negative perturbation.

Viscosity, surface tension, and motor activity affect the growth rate, ω_m , at $O(\lambda^2)$. In short, one obtains from the radial component of the force balance a fourth order ordinary

differential equation in r for $u \equiv r\delta v_{r,m}$

$$\lambda^2 \left[u^{(4)} + \frac{2}{r}u^{(3)} - \frac{(2m^2+1)}{r^2}u'' + \frac{(2m^2+1)}{r^3}u' + \frac{m^2(m^2-4)}{r^4}u \right] - \left[u'' + \frac{1}{r}u' - \frac{m^2}{r^2}u \right] = 0, \quad (6)$$

where the primes indicate differentiation with respect to r . The four boundary conditions required are that $\delta \mathbf{v}(r=0) = 0$; that the edge of the perturbed fragment is force-free, namely $\delta \sigma_{nn}(r=1) = 0$, where the subscript n refers to direction normal to the cell fragment; and that, neglecting the viscosity of the fragment's surroundings compared with the viscosity of the cytoskeleton, the tangential shear satisfies $\delta \sigma_{tn}(r=1) = 0$, where t refers to the direction tangent to the perturbed fragment.

In the limit $\lambda \rightarrow 0$, Eq. (6) together with the four boundary is a singular perturbation problem. Following the boundary layer techniques of Ref. [22], a uniformly convergent approximation to $\delta v_{r,m}$ on the interval $0 \leq r \leq 1$, valid to $O(\lambda^2)$, is

$$\delta v_{r,m}(r, t) = r^{m-1} \left\{ B_0(t) + \lambda^2 \left[B_2(t) + C_2(t)e^{\frac{(r-1)}{\lambda}} \right] \right\}, \quad (7)$$

where $B_0(t) = \frac{m}{2}\delta R_m(t)$, is the unperturbed growth rate $A_m(t)$ calculated above which is obtained from the boundary condition $\delta \sigma_{nn}(r=1) = 0$ at leading order ($O(\lambda^{-2})$); The two other constants $B_2(t)$ and $C_2(t)$ are found, respectively, by solving $\delta \sigma_{nn}(r=1) = 0$ and $\delta \sigma_{tn}(r=1) = 0$ at next-to-leading order ($O(\lambda^0)$), giving, by way of Eq. (4), a growth rate

$$\omega_m \sim (m-1) \left\{ \frac{1}{2} + m\lambda^2 \left[\frac{\zeta \Delta \mu}{\eta k_d} - 2m \right] \right\} + O(\lambda^3). \quad (8)$$

Equation (8) shows that the stabilizing effect of viscosity is proportional to m^3 for large m . It can be easily seen that the stabilizing effect of the plasma membrane tension also scales as m^3 (as does the effect of interface tension in viscous fingering instability in the Hele-Shaw cell [21]): including membrane tension, the normal stress at the boundary satisfies a two dimensional Laplace law $\delta \sigma_{nn}(r=1) = -\frac{\gamma}{\eta k_d R_0} \delta H$, where γ is the membrane tension and where the m th mode perturbation in the membrane curvature in the (r, θ) plane (ignoring changes in curvature in the z -direction) is $\delta H = (m^2 - 1)\delta R_m \cos(m\theta)$. The contribution of the membrane tension to $A_2(t)$ is $-\lambda^2 \frac{\gamma}{\eta k_d R_0} m(m^2 - 1)$. Taking $\gamma \simeq 10^{-4}$ N/m [23] as an estimate for the membrane tension and $k_d \simeq 1$ s $^{-1}$ [24] it is clear that the stabilizing effect of membrane tension is negligible compared to that of actin viscosity.

Equation (8) further shows that the contractile effect of the motors is to stabilize the growth of perturbations, proportional to m^2 . This result depends strongly on the assumption, made for simplicity, that the filament polarization in the perturbed fragment remains everywhere radial and is not a dynamical quantity in the problem. This assumption is questionable, yet it may be valid for small m , where changes in membrane curvature are small and hence any membrane-actin filament coupling is unlikely to force a significant re-orientation of filaments at the leading edge. In any case, Eq. (8) shows that the relative contribution of myosins to ω_m is proportional to $\frac{\zeta\Delta\mu}{\eta k_d}$. Taking $\zeta\Delta\mu \simeq -10^3$ Pa [5], this is of order ~ 0.1 , and therefore small compared to the viscous contribution at $O(\lambda^2)$.

Diffusion of free actin monomers also limits the perturbation growth; however, we may consider for now that the diffusion constant, D , is such that $D \gg k_d R_0^2$, so that perturbations that are area-preserving at first order in δR , that is, for $m > 1$, do not affect the essentially spatially uniform monomer density, and hence the polymerization rate, v_p . A more careful accounting of the effect of diffusion will be considered in a future publication.

Finally, it might be experimentally useful to have an estimate of the critical value of friction, ξ_c , for which shape perturbations of a cell fragment become unstable. This critical value is defined such that for $\xi < \xi_c$, $\omega_m < 0$ and for $\xi > \xi_c$, $\omega_m > 0$. It is conceivable that one could observe the onset of growing shape perturbations by plating cell fragments on surfaces of varying degrees of adhesiveness or by culturing fragments from cells that have been mutated to weaken or strengthen the binding of integrins to the surface [25, 26]. Equation (6) can be solved numerically for different mode numbers m to find the critical value ξ where the growth rate becomes positive as a function of motor strength, $|\zeta|\Delta\mu/\eta k_d$; see Fig. 2.

The numerical estimates of ξ_c given in Fig. 2 are qualitatively consistent with the value obtained by setting $\omega_m = 0$ in the asymptotic growth rate, Eq. (8): lower modes are less stable as a function of friction and motor activity has a weak effect on the growth of shape perturbations.

In summary, we have found that large substrate friction and the pressure field created by treadmilling in an initially circular cell fragment render it linearly unstable. This instability has been analyzed here in the limit $\frac{\eta}{\xi R_0^2} \rightarrow 0$, where it shows a close correspondence to the classic viscous fingering instability in Hele-Shaw cells. We have also shown by direct calculation that the effects of membrane tension, actin viscosity, and contractile motors only

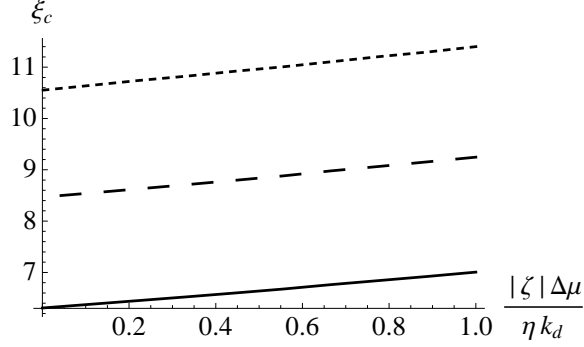


FIG. 2: Critical value of friction, ξ_c , in units of η/R_0^2 , versus $|\zeta|\Delta\mu/\eta k_d$ for $m = 2$ (solid line), $m = 3$ (long dashed line), and $m = 4$ (short dashed line). Surface tension, $\sigma/\eta k_d R_0$, is taken to be zero.

affect the flow instability at next-to-leading order in the friction. This instability has the potential to be highly relevant to the related biophysical problems of cell shape change and cell motility, given that it presents a fundamentally hydrodynamic means for cell dynamics, independent of complex biochemical signaling and, significantly, of the presence or absence of molecular motors. In future work we would like to study how the instability presented here relates to the fragment experiments of Ref. [2], in which a circular, stationary fragment could become anisotropic and motile either spontaneously or due to an external mechanical force; the reverse transition was observed in these experiments as well. In the context of the work presented here, the metastability of the fragments of Ref. [2] might be explained by their friction with the substrate being just below ξ_c for the linear instability of the mode $m = 2$. We are at present considering this possibility, by studying a possible finite amplitude instability that would couple the motile but shape-preserving mode, $m = 1$, with the shape changing modes, $m > 1$.

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